

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re the application of: Francisco, Joseph A. et al.

U.S. Serial No: 09/724,406

Filing Date: November 28, 2000

Title: *RECOMBINANT ANTI-CD30 ANTIBODIES AND USES THEREOF*

Commissioner for Patents
Washington, D.C. 20231

**DECLARATION BY DR. ROBERT F. GRAZIANO
UNDER 37 CFR 1.132 IN SUPPORT OF PUBLIC PROTEST
AGAINST US SERIAL NO. 09/724,406**

I, Dr. Robert Graziano, declare that:

1. I am presently Senior Director of Product Development at Medarex, Inc. in Bloomsbury, New Jersey. I received a B.S. in Biology from Allegheny College in 1978, and a Ph.D. in Biochemistry from Dartmouth College in 1988. My CV is attached hereto as Schedule 1.
2. I understand that the claims filed in the above-referenced US Patent Application Serial No: 09/724,406 are directed to (i) methods for treating or preventing Hodgkin's Disease in a subject by administering an antibody in a pharmaceutically acceptable carrier that immunospecifically binds CD30 and exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line, and (ii) to pharmaceutical compositions containing an antibody, which is not AC10 or HeFi-1 nor results from cleavage of AC10 or HeFi-1 with papain or pepsin, in a pharmaceutically acceptable carrier, wherein the antibody immunospecifically binds CD30 and exerts a cytostatic or cytotoxic effect on a Hodgkin's Disease cell line.
3. I am making this declaration to provide evidence that the claims in the above-referenced patent application are not patentable for lack of novelty based on the studies and prior art anti-CD30 antibodies described by Pohl et al., 1993, Int. J. Cancer 54:418-425 and Falini et al., 1992, Brit. J. Haematology 82: 38-45. I am also making this declaration to provide evidence that the claims in the above-referenced application are not patentable for lack of enablement.

In re the application of: Francisco, Joseph A. et al.
U.S. Serial No: 09/724,406
Page -2-

4. Specifically, I conducted the following *in vitro* studies using an anti-CD30 antibody, referred to as HRS-4 (also referred to as Ab1), described by Pohl et al., which showed that this antibody possesses the claimed property of exerting a cytostatic effect on HD cells, as measured in the same cytostatic cross-linking assay described in the application. In addition, the studies performed by Pohl et al. showed that HRS-4 exerts a cytotoxic effect on HD cells, as measured in the same cytotoxicity complement-mediated assay described in the application, and can be used *in vivo* to treat Hodgkin's Disease in animal models. Thus, the data described herein clearly demonstrates that the studies described by Pohl et al. using HRS-4 anticipate every element of the claimed invention.

In addition, I tested an anti-CD30 antibody, referred to as Ber-H2, described by Falini et al., in the same *in vitro* assay as HRS-4, which showed that this antibody also exerts a cytostatic effect on HD cells. However, contrary to the properties of HRS-4 described by Pohl et al., the studies performed by Falini et al. demonstrated that Ber-H2 does not treat Hodgkin's Disease *in vivo* (in patients). Thus, the data provided herein shows a lack of correlation between the claimed property of exerting a cytostatic or cytotoxic effect on HD cells and the claimed property of being useful for *in vivo* treatment of Hodgkin's disease. As such, the claimed invention in my opinion is not enabled since the application fails to provide any guidance for selecting anti-CD30 antibodies having the claimed therapeutic property of treating Hodgkin's disease and, thus, would require undue experimentation to make and use the invention as claimed.

5. The studies that I performed to generate the aforementioned data were conducted using antibodies Ber-H2, HRS-4 and a third prior art anti-CD30 antibody (as a control), referred to as AC-10. I tested these antibodies for their ability to inhibit growth of CD30, using a goat anti-mouse cross-linking antibody, in the following cell lines: L540 (Hodgkin's lymphoma derived cell line with a T cell phenotype), L428 (Hodgkin's lymphoma derived cell line with a B cell phenotype) and Karpas 299 (anaplastic large cell lymphoma derived tumor line). I employed a Ramos cell line (CD30 negative lymphoma) and the anti-murine cross-linking antibody alone in control experiments to confirm binding specificity and growth inhibition of the anti-CD30 antibodies.

6. I cultured the cell lines in flat-bottomed, 96-well tissue culture plates in a final volume of 200 μ l/well (in triplicate), and added the anti-CD30 antibodies to the cell lines to a final concentration of 2 μ g/ml and the secondary cross-linking antibody to a final concentration

In re the application of: Francisco, Joseph A. et al.
U.S. Serial No: 09/724,406
Page -3-

of 8 $\mu\text{g/ml}$. After 96 hours, I pulsed the plates with ^3H -thymidine (0.5 $\mu\text{Ci/well}$) and incubated for an additional four hours before harvesting and counting on a scintillation counter.

7. The result of this experiment was that all of the anti-CD30 antibodies inhibited growth of the CD30-expressing L540 cells (Figure 1A), L428 cells (Figure 1B), and Karpas 299 cells (Figure 1C) when cross-linked with the secondary antibody, as shown by a decreased uptake of ^3H -thymidine. The cross-linking goat anti-mouse antibody alone did not mediate this growth inhibition. Growth of the CD30 negative lymphoma line, Ramos, was not affected by any of the treatments (Figure 1D). These data demonstrate that the prior art antibodies HRS-4 and Ber-H2 can exert cytostatic or cytotoxic effects on HD cell lines *in vitro*, similarly to the antibodies claimed.

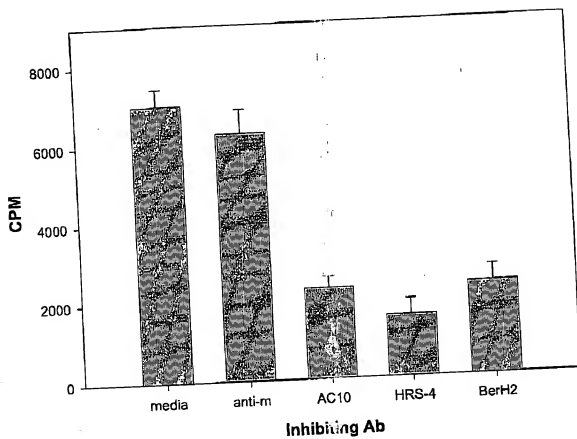
8. I understand that any willful false statements made in this declaration are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the accompanying public protest. I declare that all of the foregoing statements made of my own knowledge are true and that all statements made on information and belief are believed by me to be true.

Dated: 4/16/04

Signed: Robert Graziano
Robert Graziano, Ph.D.

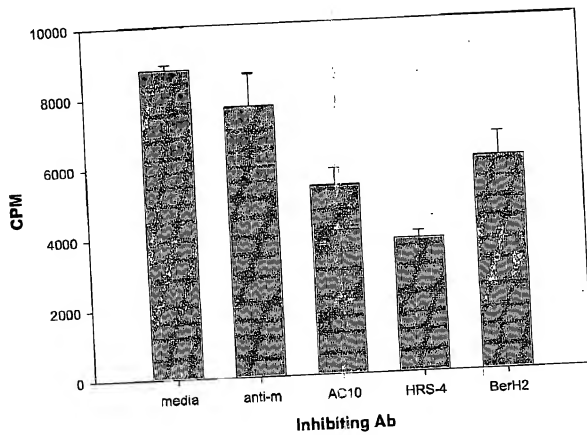
In re the application of: Francisco, Joseph A. et al.
U.S. Serial No: 09/724,406
Page -4-

Figure 1A - L540 Cells



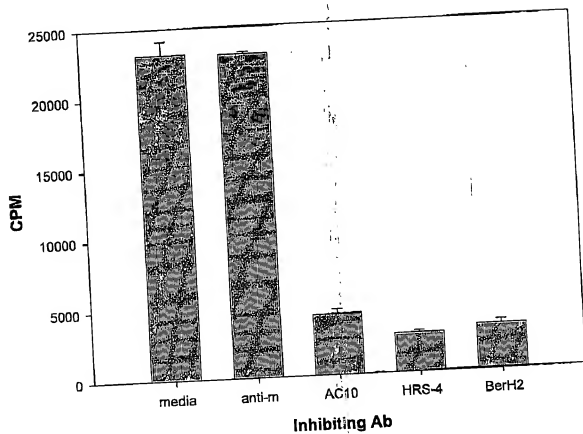
In re the application of: Francisco, Joseph A. et al.
U.S. Serial No: 09/724,406
Page -5-

Figure 1B - L428 Cells



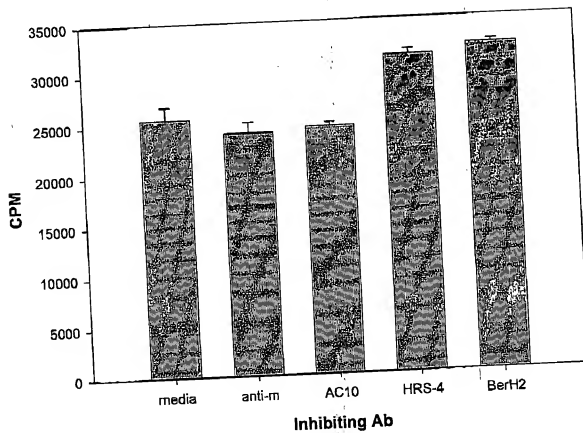
In re the application of: Francisco, Joseph A. et al.
U.S. Serial No: 09/724,406
Page -6-

Figure 1C - Kaipas 299 Cells



In re the application of: Francisco, Joseph A. et al.
U.S. Serial No: 09/724,406
Page -7-

Figure 1D - Ramos Cells



Schedule 1

CV for Robert F. Graziano, Ph.D.

PROFESSIONAL EXPERIENCE

Senior Director of Product Development

Director of Product Development

Medarex, Inc., 519 Route 173 West, Bloomsbury, NJ 08804

- Manage a team of scientists including molecular and cell biologists
- Initiate and manage collaborations with companies and academic institutions
- Create, characterize, and develop antibody-based therapeutics for treatment of cancer, autoimmune diseases, or infectious diseases utilizing Medarex's proprietary HuMAb mouse® technology
- Manage a molecular biology group responsible for support functions for Medarex, including cloning and sequencing antibody V region genes from hybridomas
- Responsible for an intermediate-scale antibody production and purification group

2002-present

2001-2002

Associate Director of Research and Development

Assistant Director of Research and Development

Medarex, Inc., 1545 Route 22 East, Annandale, NJ 08801

- Supervised a staff of 10 scientists
- Responsible for developing and characterizing novel bispecific antibodies
- Responsible for developing novel fusion proteins that target the human Fc receptors CD64 and CD89 in order to mediate direct tumor killing or for the development of tumor vaccines

1998-2001

1996-1998

Principal Scientist

Medarex, Inc., 1545 Route 22 East, Annandale, NJ 08801

- Responsible for the construction and characterization of chemically-linked bispecific antibodies
- Provided support for assay development, quality control, and production groups

1993-1996

Senior Staff Scientist

Medarex, Inc., 12 Commerce Ave., West Lebanon, NH

- Responsible for developing R&D efforts for the company
- Responsible for the development and clinical production of the first lot of MDX-210, a chemically-linked bispecific antibody

1992-1993

1992-1993

Adjunct Assistant Professor

Dartmouth Medical School, Department of Microbiology, Lebanon, NH

1981-1984

Research Assistant

Dartmouth Medical School, Department of Microbiology, Hanover, NH

- Generated monoclonal antibodies to cell surface receptors on human leukocytes

1979-1981

Research Assistant

Case Western Reserve University, Department of Microbiology, Cleveland, OH

EDUCATION

Postdoctoral Research Associate

Washington University School of Medicine, Department of Pathology, St. Louis, MO

- Generated and characterized murine T cell hybridomas specific for HIV-encoded proteins

1989-1992

Postdoctoral Research Associate

Dartmouth College, Department of Microbiology, Hanover, NH

- Characterized the structure and function of human Fc receptors

1988-1989

Ph.D. Biochemistry –

Dartmouth College, Microbiology/Immunology Departments, Hanover, NH (1984-1988)

- Thesis: Cytotoxic trigger molecules on human myeloid cells

June, 1988

B.S. Biology –

Allegheny College, Meadville, PA (1974-1978)

June, 1978

PROFESSIONAL AFFILIATIONS

American Association of Immunologists (AAI)

PUBLICATIONS

1. Ball, E.D., Graziano, R.F., Shen, L. and Fanger, M.W. Monoclonal antibodies to novel myeloid antigens reveal human neutrophil heterogeneity. *PNAS* 79:5374, 1982.
2. Graziano, R.F., Ball, E.D. and Fanger, M.W. The expression and modulation of human myeloid-specific antigens during differentiation of the HL-60 cell line. *Blood* 61:1215, 1983.
3. Ball, E.D., Graziano, R.F. and Fanger, M.W. A unique antigen expressed on myeloid cells and acute leukemia blast cells defined by a monoclonal antibody. *J. Immunol.* 130:2937, 1983.
4. Ball, E.D., Graziano, R.F., Pettengill, O.S., Sorenson, G.D. and Fanger, M.W. Monoclonal antibodies reactive with small cell carcinoma of the lung. *J. Natl. Can. Inst.* 72:593, 1984.
5. Maliszewski, C.R., Ball, E.D., Graziano, R.F. and Fanger, M.W. Isolation and characterization of My23, a myeloid cell-derived antigen reactive with the monoclonal antibody AML-2-23. *J. Immunol.* 135:1929, 1985.
6. Manjunath, R., Graziano, R.F., and Green, W.R. The specificity of H-2 restricted cytotoxic T lymphocytes directed to AKR/Gross leukemia virus induced tumors. *J. Immunol.* 136:2271, 1986.
7. Green, W.R. and Graziano, R.F. Cytolytic T lymphocyte-defined retroviral antigens on normal cells: encoding by the Akv-1 proviral locus. *Immunogenetics* 23:106, 1986.
8. Graziano, R.F. and Fanger, M.W. The use of Ig-bearing hybridomas as target cells to detect trigger molecules on human monocytes: The high affinity FcR for IgG specifically initiates monocyte-mediated cytotoxicity. *J. Immunol.* 138:945, 1987.
9. Graziano, R.F. and Fanger, M.W. Fc γ RI and Fc γ RII on monocytes and granulocytes are cytotoxic trigger molecules for tumor cells. *J. Immunol.* 139:3536, 1987.
10. Fanger, M.W., Shen, L., Graziano, R.F. and Guyre, P.M. Cytotoxicity mediated by human Fc receptors for IgG. *Immunol. Today* 10:92, 1989.
11. Graziano, R.F., Looney, R.J., Shen, L. and Fanger, M.W. Fc γ R mediated killing by eosinophils. *J. Immunol.* 142:230, 1989.
12. Shen, L., Graziano, R.F. and Fanger, M.W. The functional properties of Fc γ RI, II and III on myeloid cells: A comparative study of killing of erythrocytes and tumor cells mediated through the different Fc receptors. *Mol Immunol.* 26:959, 1989.
13. Guyre, P.M., Graziano, R.F., Vance, B.A., Morganelli, P.M. and Fanger, M.W. Monoclonal antibodies define two epitopes which trigger Fc γ RI function. *J. Immunol.* 143:1650, 1989.
14. Graziano, R.F., Erbe D.V. and Fanger, M.W. The mechanisms of antibody-dependent killing mediated by lymphoid and myeloid cells are distinct based on different divalent cation requirements. *J. Immunol.* 143:3894, 1989.

15. Fanger, M.W., Graziano, R.F. Shen, L. and Guyre, P.M. Fc γ R in cytotoxicity exerted by mononuclear cells. *Chem. Immunol.* 47:214, 1989.
16. Rigby, W.F.C., Waugh, M. and Graziano, R.F. Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25-dihydroxyvitamin D₃. *Blood* 76:189, 1990.
17. Levy, P.C., Looney, R.J., Shen, L., Graziano, R.F., Fanger, M.W., Roberts, N.J. Jr., Ryan, D.H. and Utell, M.J. Human alveolar macrophage FcR mediated cytotoxicity: studies contrasting heteroantibody versus conventional antibody mediated target cell lysis. *J. Immunol.* 144:3693, 1990.
18. Erbe, D.V., Collins, J.E., Shen, L., Graziano, R.F. and Fanger, M.W. The effect of cytokine on the expression and function of Fc receptors for IgG on human myeloid cells. *Mol. Immunol.* 27:57, 1990.
19. Graziano, R.F. and Allen, P.M. Enhancing the immunogenicity of a permissive binding T cell epitope derived from the SIV encoded negative regulatory factor. *J. Immunol.* 149:556, 1992.
20. Fanger, M.W., Graziano, R.F., and Guyre, P.M. Production and use of anti-FcR bispecific antibodies. *Immunomethods* 4:72, 1994.
21. Graziano, R.F., Somasundaram, C., and Goldstein, J. Production of bispecific antibodies. In *Bispecific Antibodies*. Edited by Michael W. Fanger. 1995.
22. Graziano, R.F., Tempest, P.R., While, P., Keler, T., Deo, Y., Ghebremariam, H., Coleman, K., Pfefferkorn, L.C., Fanger, M.W., and Guyre, P.M. Construction and characterization of a humanized anti- \square -Ig receptor type I (Fc \square RI) monoclonal antibody *J. Immunol.* 155:4996, 1995.
23. Valone, F.H., Kaufman, P.A., Guyre, P.M., Lewis, L.D., Memoli, V., Deo, Y., Graziano, R.F., Fisher, J.L., Meyer, M., Mrozek-Orlowski, M., Wardwell, K., Guyre, V., Morley, T.L., Arrizu, C., and Fanger, M.W. Phase I/II trial of bispecific antibody MDX-210 in patients with advanced breast or ovarian cancer that overexpress the proto-oncogene HER-2/Neu. *J. Clin. Oncol.* 14:2281, 1995.
24. Heijnen, I.A.F.M., van Vugt, M.J., Fanger, N.A., Graziano, R.F., de Wit, Ton P.M., Hofhuis, F.M.A., Guyre, P.M., Capel, P.J.A., Verbeek, J.S., and van de Winkel L.G.J. Antigen targeting to myeloid-specific human Fc γ RI/CD64 triggers enhanced antibody responses in transgenic mice. *J. Clin. Invest.* 97:331, 1996.
25. Ely, P., Wallace, P.K., Givan, A.L., Graziano, R.F., Guyre, P.M., Fanger, M.W. Bispecific-armed, IFN γ -primed macrophage-mediated phagocytosis of malignant non-Hodgkin's lymphoma. *Blood* 87:3813, 1996.
26. Liu, C., Goldstein, J., Graziano, R.F., He, J., O'Shea, J.K., Deo, Y.M., Guyre, P.M. Fc γ RI-targeted fusion proteins result in efficient presentation by human monocytes of antigenic and antagonist T cell epitopes. *J. Clin. Invest.* 98:2001, 1996.
27. Keler, T., Graziano, R.F., Mandal, A., Wallace, P.K., Fisher, J., Guyre, P.M., Fanger, M.W., Deo, Y.M. Bispecific antibody-dependent cellular cytotoxicity of HER2/neu-over-expressing tumor cells by Fc γ receptor type I-expressing effector cells. *Cancer Res.* 57:4008, 1997.

28. Goldstein, J., Graziano, R.F., Fanger, M.W. Bispecific fusion protein. In *Antibody Fusion Proteins* (S Chamow, editor) R. G. Landes Company, 1997.
29. Deo, Y.M., Graziano, R.F., Repp, R., van de Winkel, J.G.J. Clinical significance of IgG Fc receptors and Fc γ R-directed immunotherapies. *Immunol. Today* 18: 127, 1997.
30. Goldstein, J., Graziano, R.F., Sundarapandiyam, K., Somasundaram, C., Deo, Y.M. Cytolytic and cytostatic properties of an anti-human Fc γ RI (CD64) X epidermal growth factor bispecific fusion protein. *J. Immunol.* 158: 872, 1997.
31. Wallace, P.K., Keler, T., Coleman, K., Fisher, J., Vitale, L., Graziano, R.F., Guyre, P.M., Fanger, M.W. Humanized mAb H22 binds the high affinity Fc receptor for IgG (Fc γ RI), blocks phagocytosis, and modulates receptor expression. *J. Leukocyte Biol.* 62:469, 1997.
32. Graziano, R.F., Goldstein, J., Sundarapandiyam, K., Somasundaram, C., Keler, T., Deo, Y.M. Targeting tumor cell destruction with CD64-directed bispecific fusion proteins. *Cancer Immunol. Immunotherapy* 45:124, 1997.
33. Fanger, N.A., Voightlaender, D., Liu, C., Swink, S., Wardwell, K., Fisher, J., Graziano, R.F., Pfefferkorn, L.C., Guyre, P.M. Characterization of expression, cytokine regulation, and effector function of the high affinity IgG receptor Fc gamma RI (CD64) expressed on human blood dendritic cells. *J. Immunol.* 158:3090, 1997.
34. Deo, Y.M., Sundarapandiyam, K., Keler, T., Graziano, R.F. Bispecific molecules directed to the Fc receptor for IgA (Fc γ RI, CD89) and tumor antigens efficiently promote cell-mediated cytotoxicity of tumor targets in the whole blood. *J. Immunol.* 160:1677, 1998.
35. Somasundaram, C., Sundarapandiyam, K., Keler, T., Deo, Y.M., Graziano, R.F. Development of a trispecific antibody conjugate that directs two distinct tumor-associated antigens to CD64 on myeloid effector cells. *Hum Antibodies* 9:47-54, 1999.
36. Keler, T., Wallace, P.K., Vitale, L.A., Russoniello, C., Sundarapandiyam, K., Graziano, R.F., Deo, Y.M. Differential effect of cytokine treatment on Fc alpha receptor I- and Fc gamma receptor I-mediated tumor cytotoxicity by monocyte-derived macrophages. *J. Immunol.* 164:5746-52, 2000.
37. Stockmeyer, B., Dechant, M., van Egmond, M., Tutt, A.L., Sundarapandiyam, K., Graziano, R.F., Repp, R., Kalden, J.R., Gramatzki, M., Glennie, M.J., van de Winkel, J.G., Valerius, T. Triggering Fc alpha-receptor I (CD89) recruits neutrophils as effector cells for CD20-directed antibody therapy. *J. Immunol.* 165:5954-61, 2000.
38. Keler, T., Guyre, P.M., Vitale, L.A., Sundarapandiyam, K., van de Winkel, J.G., Deo, Y.M., Graziano, R.F. Targeting weak antigens to CD64 elicits potent humoral responses in human CD64 transgenic mice. *J. Immunol.* 165:6738-42, 2000.

39. Sundarapandiyar, K., Keler, T., Behnke, D., Engert, A., Barth, S., Matthey, B., Deo, Y.M., Graziano, R.F. Bispecific antibody-mediated destruction of Hodgkin's lymphoma cells. *J. Immunol. Methods.* 248:113-23, 2001.
40. Guyre, C.A., Keler, T., Swink, S.L., Vitale, L.A., Graziano, R.F., Fanger, M.W. Receptor modulation by Fc gamma RI-specific fusion proteins is dependent on receptor number and modified by IgG. *J. Immunol.* 167:6303-11, 2001.
41. Walsh, M.C., Banas, J.A., Mudzinski, S.P., Preissler, M.T., Graziano, R.F., Gosselin, E.J. A two component modular approach for enhancing T-cell activation utilizing a unique anti-Fc gamma RI-streptavidin construct and microspheres coated with biotinylated-antigen. *Biomol. Eng.* 20:21-33, 2003.
42. Borchmann, P., Treml, J.F., Hansen, H., Gottstein, C., Schnell, R., Staak, O., Zhang, H., Davis, T., Keler, T., Diehl, V., Graziano, R.F., and Engert, A. The human anti-CD30 antibody 5F11 shows in vitro and in vivo activity against malignant lymphoma. *Blood*: 102: 3737 – 3742, 2003.

PATENTS
(List limited to published US filings)

- US 6,682,928 Cells expressing anti-Fc receptor binding components
- US 6,410,690 Therapeutic compounds comprised of anti-Fc receptor antibodies
- US 6,395,272 Therapeutic compounds comprised of anti-Fc receptor antibodies
- US 6,365,161 Therapeutic compounds comprised of anti-Fc receptor binding agents
- US 6,303,755 Therapeutic multispecific compounds comprised of anti-Fc α receptor antibodies
- US 6,270,765 Therapeutic compounds comprised of anti-Fc receptor antibodies
- US 6,193,966 Therapeutic multispecific compounds comprised of anti-Fc α receptor antibodies
- US 5,922,845 Therapeutic multispecific compounds comprised of anti-Fc α receptor antibodies
- US 5,837,243 Therapeutic compounds comprised of anti-Fc receptor antibodies
- US 20040006215 Human monoclonal antibodies against CD30
- US 20040005318 Methods of treatment using CTLA-4 antibodies
- US 20020032312 Therapeutic compounds comprised of anti-Fc receptor antibodies
- US 20010014328 Therapeutic multispecific compounds comprised of anti-Fc α receptor antibodies